Oxitec EUP Office Director Briefing

Our plan is to discuss background, risk assessment, and terms. Most of this has already been sent to management and reviewed so please interrupt so we can focus on areas of most concern. Thanks!

EUP Background

Oxitec Ltd., (Oxitec or the applicant) requested an Experimental Use Permit (EUP) under FIFRA section 5 for a new end-use product OX5034 containing a variant of the new active ingredient tetracycline-repressible transactivator (tTAV-OX5034) protein, a variant of the new inert ingredient DsRed2 protein (DsRed2-OX5034), and the genetic material (vector pOX5034) necessary for their production in OX5034 *Aedes aegypti* (yellow fever mosquito). Oxitec requested this EUP to evaluate whether the product is efficacious in suppressing naturally occurring *Ae. aegypti* populations under field conditions.

OX5034 is described as a species-specific female-lethal trait that results in emergence of all-male progeny in the absence of tetracycline in the larval diet. The pesticidal effect of OX5034 is species-specific as it only affects the reproductive success of *Ae. aegypti* through mating between OX5034 *Ae. aegypti* males and *Ae. aegypti* females that are already present in the release area. OX5034 homozygous males alone will be released into the environment. Only female offspring from OX5034 matings are killed, while OX5034 hemizygous males survive to pass on the OX5034 female-lethal trait further. Unlike female mosquitoes, male mosquitoes do not bite humans. With continued field releases of OX5034 homozygous males, the *Ae. aegypti* population in the treatment area is thought to progressively decline due to the reduced number of females emerging each consecutive generation. In addition, OX5034 also expresses DsRed2-OX5034, a variant of the DsRed fluorescent protein form *Discosoma* spp., that allows for the visual identification of OX5034 hemizygous larvae collected from the field. A different transgenic mosquito developed by Oxitec, OX513A, is not covered under the current EUP application. With this application, Oxitec provided the experimental design for mosquito releases under the EUP. EPA evaluated this in its document "Review of Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 *Ae. aegypti* Mosquitoes Decision #549240; Submission #1047971," which is available in the www.regulations.gov docket established for this action ([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2019-0274"]). Oxitec requested a 24-month permit for a cumulative area of 6,600 acres, which will be divided into multiple treatment and control areas within Monroe, Co., Florida and Harris, Co., Texas.

STATE	ACRES
Florida	3120
Texas	0
TOTAL (YEAR 2020-2021)	**3120**
Florida	3120
Texas	360
TOTAL (YEAR 2021-2022)	**3480**
OVERALL PERMIT TOTAL	**6,600**

Under the EUP, Oxitec is planning to test the efficacy of the product by deploying OX5034 mosquito eggs and adult males in the treatment areas. For egg releases, a known quantity of OX5034 eggs will be released in mosquito rearing boxes. Importantly, as described in Unit II.A.2, only male OX5034 mosquitoes will emerge from these eggs, no female OX5034 mosquitoes will be released. Mosquito rearing boxes will be physically isolated from the public whenever possible, or otherwise located discretely and out of public view. In the case of adult OX5034 male releases, known quantities of adult males will be released from containers either from a vehicle or on foot.

Monitoring and mosquito sampling will be done weekly in the treatment and control areas to monitor the adult mosquito population and to collect eggs. The egg collections will allow for evaluation of larvae resulting from male OX5034 mosquito matings, which will be used to determine how well the product works for mosquito control and provide monitoring to confirm no female OX5034 mosquitoes. Additional monitoring will also occur once releases have ceased to ensure that the OX5034 traits disappear from the male mosquitoes in the EUP locations as is expected.

Human Health and Environmental Risk Assessment

In assessing the risk to human health and the environment from the limited releases of OX5034 mosquitoes in Monroe, Co., Florida and Harris, Co., Texas over two (2) years, several key factors played a significant role.

- Only male OX5034 mosquitoes will be released into the environment. Because male mosquitoes do not feed on humans (they do not bite), they do not pose a human health risk.
- Female mosquitoes feed on human blood, but only once they become adults.
- Oxitec's OX5034 female mosquitoes do not survive to become adults without tetracycline. Tetracycline acts as an antidote to the OX5034 female mosquito-lethal trait.
- EPA evaluated penetrance of the OX5034 female-lethal trait.
    - Penetrance for the OX5034 mosquitoes refers to the proportion of female insects that die before reaching adulthood, i.e. does it consistently work. EPA found that it does.
- EPA evaluated human health risk of OX5034 mosquitoes.
    - A determination of the toxicity and allergenicity of the two substances in Oxitec's OX5034 mosquitoes that 1) kill female mosquitoes, tTAV-OX5034, and 2) allow trained personnel to identify OX5034 via fluorescence, DsRed2-OX5034, has not been made.
    - However, because no OX5034 female mosquitoes are being released or are expected to emerge in the environment, exposure is negligible and therefore, so is the potential risk from tTAV-OX5034 and DsRed2-OX5034 (Risk = Exposure x Hazard).
- EPA evaluated introgression risk.

- Introgression for the OX5034 mosquitoes refers to the movement of background traits from the non-GE portion of the OX5034 mosquito genome to local mosquitoes, i.e. will releases of OX5034 mosquitoes increase the ability of wild mosquitoes in the release area to vector/transmit disease, result in larger populations numbers, or result in more robust mosquitoes. EPA found this impact is unlikely. As part of this analysis, EPA collaborated with the United States Centers for Disease Control and Prevention (CDC) in reviewing laboratory data, a meta-analysis, and rationale submitted by the applicant comparing the vectorial capacity of OX5034 mosquitoes to that of wild mosquitoes.

- EPA evaluated the risk of OX5034 mosquitoes to non-target organisms (bats, amphibians, etc.).
  - No direct adverse effects due to consumption of OX5034 males by non-target organisms is expected based on acute oral toxicity studies and bioinformatics analyses.
  - *Ae. aegypti* mosquitoes (of which OX5034 mosquitoes are) are not a sole or critical food source for non-target organisms, so no indirect adverse effects are expected should there be a decrease in the local mosquito population.

Based on the above factors and analyses discussed in EPA's science assessment, EPA determined that there will be no unreasonable adverse effects to humans or the environment as a result of the experimental permit to release Oxitec's OX5034 male mosquito. Below are EPA's risk conclusions for the human health and environmental risk assessment, which can also be found in Unit III, "Human Health & Environmental Risk Conclusions:"

EPA has reviewed the OX5034 manufacturing process detailing the production and quality assurance processes used in the development and manufacture of OX5034 mosquitoes, associated standard operating procedures, and other pertinent information characterizing OX5034 mosquitoes on a genetic and phenotypic level. EPA determined this information to be adequate to support a finding of no unreasonable adverse effects to man and the environment during the proposed EUP.

EPA has determined that there will be no unreasonable adverse effects for humans as a result of the experimental permit to release *Ae. aegypti* OX5034 male mosquitoes provided such releases do not take place within 400 m of commercial citrus growing areas or wastewater treatment sites due to considerations regarding the impact of environmental sources of tetracyclines on female OX5034 mosquito survival. A compilation of release recapture studies around the world found that most *Ae. aegypti* are recovered within 20 m to 50 m of the release point, with a small percentage found 170 m but generally not more than 200 m from the release point. Therefore, a restriction of 500 m from potential sources (200 m for released OX5034 males + 200 m for mated *Ae. aegypti* females + 100 m of additional buffer) provides a conservative buffer zone. The human health assessment considered data provided on the mammalian toxicity and allergenicity of the tTAV-OX5034 (active ingredient) and DsRed2-OX5034 (inert ingredient) proteins and the potential routes through which humans may be exposed to these substances as a result of OX5034 application. While no determination has been made on the potential of either protein to pose mammalian hazard, the human health risk was found to be negligible, as exposure to female mosquitoes carrying these traits was determined to be negligible given that the penetrance of the

tTAV-OX5034 lethal trait was shown to be 100% in female mosquitoes and the restrictions on access to potential tetracycline sources.

EPA has determined that there will be no unreasonable adverse effects for humans or the environment due to introgression of OX5034 background strain genetics into the local *Ae. aegypti* population. EPA evaluated OX5034 mosquitoes for key traits that could increase the ability of mosquitoes to transmit disease, result in larger populations numbers, or result in more robust mosquitoes. Based on a combination of laboratory data, meta-analyses, and a review of the scientific literature, EPA finds it is unlikely that the local mosquito population would pose any increased risk to humans or the environment as a result of releases of OX5034 mosquitoes and introgression of OX5034 background strain genetics.

EPA has also determined that no unreasonable adverse effects are anticipated for non-target organisms as a result of the experimental permit to release *Ae. aegypti* OX5034 male mosquitoes. No direct adverse effects due to consumption of OX5034 males by non-target organisms is expected based on acute oral toxicity studies and bioinformatics analyses. There are also no indirect adverse effects anticipated from reduction in *Ae. aegypti* as a food source should the release of OX5034 mosquitoes successfully reduce the local *Ae. aegypti* population. In the case of *Ae. aegypti*, their status as invasive species and their oviposition choice behavior makes it less likely that they serve an integral role in newly invaded ecosystems. Additionally, *Ae. aegypti* are regularly subjected to other control methods such as insecticide treatment and source reduction and it is therefore unlikely any predator species or plant is dependent on *Ae. aegypti* presence.

EUP Terms

- You will immediately notify (within 24 hours) the EPA of any findings from the experimental uses that have a bearing on safety (i.e., the EPA requires reporting of any adverse effects from the use of or exposure to pesticides). This includes, among other findings, any violation of the required 500 meter release distances from citrus orchards or municipal sewage treatment plants. You will also keep records of production, distribution, and performance, and make the records available on request to any authorized officer or employee of the EPA.

1) Releases must not occur within 500 meters of sewage treatment facilities and any farm producing citrus crops.

2) Oxitec must conduct continuous weekly monitoring for fluorescent larvae at release sites as indicated in the section G experimental program (sections 5.2.6.1 and 5.9.4.1). From the reared field-collected individuals, Oxitec must determine the presence of the genetic cassette (vector pOX5034) in a minimum of 150 non-fluorescent adult female *Ae. aegypti* following the standard operating procedures QD-R-00109 or QD-R-00108 once per month. If at any time during the course of the EUP Oxitec finds female individuals containing the OX5034 genetic construct surviving to ~~adults~~ adulthood, Oxitec must take the following remediation actions: Immediately cease releases of all OX5034 mosquitoes, as soon as practicable apply adulticide and larvicide pesticides to the treated area where the surviving females were detected ~~from~~ and continue to monitor for the presence of the

OX5034 genetic construct in female *Ae. aegypti* until OX5034 mosquitoes are no longer found for at least two successive mosquito generations, a minimum of 10 weeks." EPA may require additional applications of adulticides and larvicides if fluorescent mosquitoes continue to be found in the treated area after the initial detection.

3) If evidence of invasive *Aedes* spp. or arboviruses principally vectored by *Ae. aegypti* becoming established in the UK, colony related testing will be required.

4) As indicated in the section G experimental program (sections 5.3.1 and 5.10), Oxitec must conduct post-release monitoring until no fluorescent OX5034 mosquitoes have been found for at least two successive generations, a minimum of 10 consecutive weeks.